styryl ring, and the styryl group has been attached at the 4- or sometimes the 2-position on the quinoline ring.[2]

We have prepared isomers of the active 4-(4-aminostyryl)quinoline by changing the position of the attachment of the aminostyryl group on the quinoline or changing the position of the amino group on the styryl ring. All of the compounds listed in Table I were prepared by reduction of the corresponding nitro compounds with stannous chloride in concentrated hydrochloric acid at 80–110°, and were recrystallized repeatedly before analysis. The three compounds having a 4-aminostyryl group on the benzene ring of the quinoline appear to be more toxic than the others in rats, but not in KB cell cultures, and did not show superior antitumor activity against the Walker 256 tumor. The two compounds containing a 4-amino group on a styryl group attached at the 4-position on the pyridine ring were most effective in cell culture inhibition and strongly inhibited growth of the Walker tumor.

(2) C. T. Bahner, C. Cook, J. Dale, J. Fain, F. Hannan, P. Smith, and J. Wilson, *J. Org. Chem.*, **23**, 1060 (1958).

6-(5-Nitro-2-furyl)-*as*-triazine-3,5(2H,4H)-dione. A Potential Urinary Tract Antibacterial

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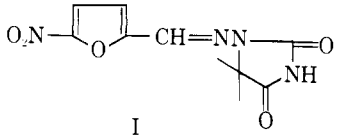
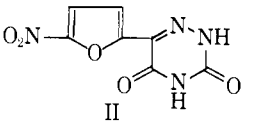
The significant utility of nitrofurantoin[1] for the control of bacterial infections of the urinary tract[2] has inspired the search for other nitrofuran structures for this application. The criteria considered significant include: resistance to metabolic degradation as evidenced by a high level of renal excretion, an adequate antibacterial spectrum, an adequate therapeutic index, and low incidence of emesis.

Incorporation of the hydrolytically vulnerable azomethine linkage of nitrofurantoin (I) in an imidic 1,2,4-triazine seemed likely to aid resistance to metabolic degradation and yet permit high kidney clearance. 6-(5-Nitro-2-furyl)-*as*-triazine-3,5(2H,4H)-dione (II), fitting these tentative requirements, was prepared and evaluated.



I



II

2-Furoylformic acid semicarbazone was treated with sodium alkoxide in propylene glycol to yield 6-(2-furyl)-*as*-triazine-3,5(2H,4H)-dione. This furan derivative was nitrated in acetic anhydride to give II.

Some biological observations made on II are presented in Tables I–III in comparison with nitrofurantoin. These data indicate II to be a significant candidate for further investigation as a urinary tract antibacterial agent.

TABLE I

SENSITIVITY OF BACTERIA ISOLATED FROM URINARY TRACT INFECTIONS TO NITROFURANTOIN SODIUM (I) AND II[a]

Bacterial species	I: No. sensitive / No. tested	I: Limits of zones[b]	II: No. sensitive / No. tested	II: Limits of zones[b]
Escherichia coli	26/26	9–24	25/26	0–22
Proteus sp.	1/14	0–10	0/14	0
Aerobacter	12/15	0–15	11/15	0–14
Pseudomonas sp.	0/16	0	1/10	0–9
Alcaligenes faecalis	1/2	0–11	1/2	0–12
Staphylococcus aureus	6/6	17–22	6/6	13–23
Streptococcus (group D)	7/8	0–21	8/8	14–24

[a] Bacteriological data supplied by Dr. J. O'Connor. [b] Impregnated paper disks (30 γ). Zone diameters in mm. include the 6-mm. disk, except negative reactions are recorded as 0. Averages of 6 determinations.

TABLE II

URINARY EXCRETION OF I AND II[a]

Animal	Dose, mg./kg.	% excreted in urine: I		II	
Mouse	10	22.3[b]	(25.3)[c]	21.3[b]	(23.0)[c]
Rat	10	44.7	(42.3)	50.2	(42.8)
Monkey	10	16.0	(16.3)	18.3	(21.7)

[a] These data supplied by Dr. R. Bender. [b] Per cent of oral dose as determined by antibacterial assay; 24-hr. urine collection. [c] Per cent of oral dose as determined by ultraviolet spectroscopy; 24-hr. urine collection. Ultraviolet curves of excreted urine resemble that for drugs administered.

TABLE III

ACUTE TOXICITY[a]

Animal	I	II
	Oral median lethal dose, mg./kg.	
Mouse	605	940
Rat	981	950
	Median emetic dose, mg./kg.	
Dog	25	100

[a] Toxicological data supplied by Dr. A. R. Borgmann.

Experimental

2-Furoylformic Acid Semicarbazone.—2-Furoyl cyanide[3] (44 g.) was hydrolyzed with concentrated hydrochloric acid to 2-furoylformic acid by the method of Fischer.[3] The crude acid was dissolved in 50 ml. of ethanol and added to a solution of 44 g. of semicarbazide hydrochloride in 450 ml. of water. The solid semicarbazone was filtered, washed with water, alcohol, and ether. The crude product (40.5 g.) was purified by solution in a mixture of 1500 ml. of water and 100 ml. of concentrated ammonium hydroxide and treating with charcoal. Acidification of the filtered solution gave white, felted needles which were filtered and dried at 110°; yield 33 g. (46%), m.p. 174° dec. (Fisher–Johns, corrected). An analytical sample was recrystallized from 50% aqueous 2-propanol.

Anal. Calcd. for $C_7H_7N_3O_4$: C, 42.64; H, 3.58; N, 21.32. Found: C, 42.79, 42.68; H, 3.69, 3.67; N, 21.12, 21.25.

6-(2-Furyl)-*as*-triazine-3,5(2H,4H)-dione.—2-Furoylformic acid semicarbazone (20 g.) in 450 ml. of propylene glycol was mixed with a sodium ethoxide solution prepared from 7.5 g. of sodium in 150 ml. of absolute ethanol and refluxed for 24 hr.

(1) K. Hayes, U. S. Patent 2,610,181 (1952).

(2) (a) C. Norfleet, Jr., P. Beamer, and H. Carpenter, Transactions, Southeast Section of the American Urological Association, Boca Raton, Fla., 1952, p. 26; (b) S. Mintzer, E. Kadison, W. Shlaes, and O. Felsenfeld, *Antibiot. Chemotherapy*, **3**, 151 (1953); (c) B. Waisbren, *A.M.A. Arch. Internal Med.*, **101**, 397 (1958).

(3) (a) E. Fischer and F. Brauns, *Ber.*, **46**, 892 (1913). (b) To avoid the use of HCN, 2-furoyl cyanide can be conveniently prepared in 48% yield from the acid chloride and cuprous cyanide by the procedure of T. Oakwood and C. Weisgerber for benzoyl cyanide ("Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 112).

The solvents were removed by vacuum distillation. The residual yellow solid was dissolved in 110 ml. of hot water, cooled by adding 100 g. of ice, and acidified with 30 ml. of concentrated HCl. The white solid product was filtered, washed well with water, and dried to constant weight; yield 16.1 g. (88%), m.p. 318–320°, sealed tube (copper block, corrected). This material was suitable for nitration. For purification for analysis the product was dissolved in dilute ammonium hydroxide, treated with charcoal, and reprecipitated with acid. The melting point was unchanged.

Anal. Calcd. for $C_7H_5N_3O_4$: C, 46.93; H, 2.81; N, 23.46. Found: C, 46.95, 46.95; H, 2.84, 2.86; N, 23.60, 23.63.

6-(5-Nitro-2-furyl)-*as*-triazine-3,5(2H,4H)-dione (II).—A nitration mixture was prepared by slowly mixing 8.4 ml. of 70% nitric acid containing 2 drops of concentrated sulfuric acid and 38 ml. of acetic anhydride with cooling below 25°. Solid, powdered 6-(2-furyl)-*as*-triazine-3,5(2H,4H)-dione (7.2 g.) was added in small increments with stirring and cooling to keep the temperature at 25–30°. After 1 hr., 18 g. of fused potassium acetate was added and the temperature held at 45–50° for 1 hr. The thick nitration mixture was poured into 250 ml. of ice water. The bright yellow solid was filtered, washed with water, and dried at 110°. The yield was 6.4 g. (71%), m.p. 330–331° (sealed tube, copper block, corrected). For analysis it was recrystallized from a 1:5 mixture of dimethylformamide and nitromethane and from acetonitrile; m.p. 332–333° (as above).

Anal. Calcd. for $C_7H_4N_4O_5$: C, 37.51; H, 1.80; N, 25.00. Found: C, 37.71; H, 1.92; N, 24.80, 24.88.

II is weakly acidic; ultraviolet absorption (in water) showed λ 365 mμ (ϵ_{max} 18,100); solubility in water at 25°, pH 5.5, is 45 mg./l.

Acknowledgment.—Analytical determinations were made by G. Ginther, M. Tefft, and G. Gustin.

New Compounds

Synthesis of Tripeptides of α-Aminoisobutyric Acid[1a]

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The unnatural, unmetabolizable amino acid, α-aminoisobutyric acid, is a useful model for the study of biological transport of neutral amino acids.[2] We have synthesized two new tripeptides of this amino acid, glycyl-α-aminoisobutyryl-L-alanine and glycyl-α-aminoisobutyryl-L-valine, for use as model substrates in the study of peptide transport. The uptake of glycyl-α-aminoisobutyryl-1-C^{14}-L-alanine by *Lactobacillus casei* 7469 has been reported elsewhere.[3]

The synthesis of carbobenzoxyglycyl-α-aminoisobutyric acid ethyl ester and the corresponding acylated dipeptide by the acid chloride coupling method has been reported by Bergmann, *et al.*[4] We have used the carbodiimide coupling method[5] to make the same compounds, precursors of our tripeptides.

Experimental[6]

Carbobenzoxyglycyl-α-aminoisobutyric Acid Ethyl Ester.—To a solution of 10.46 g. (0.05 mole) of carbobenzoxyglycine prepared by the usual method, 8.38 g. (0.05 mole) of α-aminoisobutyric acid ethyl ester hydrochloride, and 8 ml. (0.055 mole) of triethylamine in 200 ml. of methylene chloride was added 20.64 g. (0.10 mole) of dicyclohexylcarbodiimide. The mixture was stirred for 13 hr. at room temperature, then allowed to stand for 17 hr. at room temperature. After the addition of 1.0 ml. of glacial acetic acid, the mixture was allowed to stand in ice for 1 hr. and the dicyclohexylurea was removed by filtration. The filtrate was washed with 1 *N* HCl, 1 *N* $NaHCO_3$, and water, and dried (Na_2SO_4). Removal of methylene chloride *in vacuo* yielded 14.45 g. of crude product, m.p. 72.5–75.5°. Recrystallization from ethyl acetate–petroleum ether (b.p. 40–56°) gave 11.80 g. (73.1% yield), m.p. 80.5–83°, lit.[4] m.p. 84°. The radioactive, protected dipeptide, labeled with C^{14} in the carboxyl group of α-aminoisobutyric acid,[7] was prepared by a virtually identical procedure, as described in detail in the dissertation.[1b]

Carbobenzoxyglycyl-α-aminoisobutyric Acid.—To a solution of 4.84 g. (0.015 mole) of the above protected dipeptide in a mixture of 20 ml. of water and 40 ml. of dioxane was added 2.25 ml. (0.0225 mole) of 10 *N* NaOH. The solution was stirred at 4° for 5 hr.; 4.5 ml. of 5 *N* HCl was added, giving pH 4.5; the reaction mixture was stored at 4° for 15 hr., concentrated *in vacuo*, and the crude product was recrystallized from hot water, 3.67-g. yield (83.3%), m.p. 155–158.5°, lit.[4] m.p. 155.5°. The C^{14}-labeled acylated dipeptide was prepared by a similar procedure.[1b]

Carbobenzoxyglycyl-α-aminoisobutyryl-L-alanine Benzyl Ester. Azide Method.—A modification of the procedure of Erlanger and Brand[8] was employed. To 12.89 g. (0.04 mole) of carbobenzoxyglycyl-α-aminoisobutyric acid ethyl ester dissolved in 80 ml. of hot absolute ethanol was added 5.01 g. (0.1 mole) of hydrazine hydrate. The mixture was refluxed for 2 hr. No crystals appeared on cooling to room temperature. An additional 10.0 g. (0.2 mole) of hydrazine hydrate was added; the mixture was refluxed for 2 hr. longer and left in the cold overnight. The solvent was removed *in vacuo;* the residue was resuspended in ethyl ether and the solvent was removed *in vacuo* three more times to yield 12.87 g. of crude product. Recrystallization from absolute ethanol–ethyl ether yielded 7.91 g. (64.0%) of the hydrazide, m.p. 119–121.5°.

The protected tripeptide was prepared from 3.09 g. (0.01 mole) of the above hydrazide [converted to the azide by treatment with 0.759 g. (0.11 mole) of sodium nitrite] and an ethereal solution of L-alanine benzyl ester [freshly prepared from 4.72 g. (0.014 mole) of L-alanine benzyl ester benzenesulfonate and 2.3 ml. (0.016 mole) of triethylamine]. After 55 hr. standing at room temperature, a white fibrous product was collected by filtration, washed, dried in the usual manner, and chilled to −12° to yield a second crop. Recrystallization from hot absolute ethanol yielded 2.36 g. (51.7%) of protected tripeptide, m.p. 159.5–163°, $[\alpha]^{26}D$ −22.9° (*c* 1, ethanol).

Anal. Calcd. for $C_{24}H_{29}N_3O_6$: C, 63.28; H, 6.42; N, 9.23. Found: C, 63.09; H, 6.10; N, 9.31.

Carbodiimide Method.—To a solution of 1.47 g. (0.005 mole) of carbobenzoxyglycyl-α-aminoisobutyric acid and 0.005 mole of L-alanine benzyl ester benzenesulfonate and 0.8 ml. (0.0055 mole) of triethylamine in 150 ml. of acetonitrile was added 2.06 g. (0.01 mole) of dicyclohexylcarbodiimide. The reaction mixture was stirred for 10 hr. at room temperature and allowed to stand at room temperature for 21 hr.; 1.0 ml. of glacial acetic acid was added and the product was worked up as usual, after replacement

(1) (a) From the Ph.D. Thesis of Ellen A. Young, University of Arkansas, 1963. This work was carried out while Ellen A. Young was a fellow supported by Training Grant 5T1-GM-551-03 from the United States Public Health Service. (b) Department of Radiology, University of Arkansas Medical Center, Little Rock, Ark.

(2) H. N. Christensen and J. C. Jones, *J. Biol. Chem.*, **237**, 1203 (1962).

(3) E. A. Young, D. O. Bowen, and J. F. Diehl, *Biochem. Biophys. Res. Commun.*, **14**, 250 (1964).

(4) M. Bergmann, L. Zervas, J. S. Fruton, F. Schneider, and H. Schleich, *J. Biol. Chem.*, **109**, 325 (1935).

(5) J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955).

(6) Melting points were determined on a Fisher-Johns block and are corrected. Specific rotation was determined with the aid of a 0.01° Zeiss polarimeter, using the D line of sodium. Elementary analyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y., and by Clark Microanalytical Laboratory, Urbana, Ill.

(7) Carboxyl-labeled α-aminoisobutyric acid, 1.238 mc./mmole, was obtained from Tracerlab, Inc., Waltham, Mass.

(8) B. F. Erlanger and E. Brand, *J. Am. Chem. Soc.*, **73**, 3508 (1951).